BIOLOGICAL CONTROL OF MOSQUITOES WITH THE NEMATODE, ROMANOMERMIS CULICIVORAX.

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BACKGROUND: Many areas of Florida (such as Broward, Dade, and Palm Beach counties) have witnessed high rates of urbanization in the past twenty years. This urban development has slowly encroached upon areas with naturally high numbers of mosquitoes and created new breeding habitats for these pests. Because of Florida's booming tourist trade and large urban population, there is growing sensitivity to the seasonal nuisance caused by biting adult female mosquitoes. In addition, mosquitoes are vectors for many human diseases in the world, malaria (protozoan disease) and lymphatic filariasis (nematode) with 400 million and 50 million people estimated to be carriers worldwide, respectively (6). The cosmopolitan nature of Florida necessitates a constant battle to keep potential mosquito vectors and diseased individuals separated to prevent disease outbreaks.

Fifty-seven million dollars are spent each year for mosquito control in Florida (10). Chemical control has been useful in the past, but there have been problems including pesticide resistance, economics, and environmental pollution. Because of these drawbacks there has been an increasing interest in the biological control of mosquitoes. The World Health Organization has recognized several promising agents for the control of culicids. Included in their list were mosquito pathogenic bacteria, fungi, protozoans, and the nematode, Romanomermis culicivorax (6).

 \underline{R} . $\underline{\text{culicivorax}}$ is an aquatic mermithid that occurs naturally in Louisiana and Florida $\overline{(14)}$. All mermithids are specialized obligate endoparasites of invertebrates. \underline{R} . $\underline{\text{culicivorax}}$ is host specific to the mosquito family, Culicidae, and has been reported to parasitize the larvae of at least seventy-two species of mosquitoes including the predatory and beneficial mosquito, $\underline{\text{Toxorhynchites}}$ amboinensis (2,13).

The life cycle of R. culicivorax is depicted in Fig. 1. Eggs of R. culicivorax which contain preparasites (J2) or coiled preparasites in the soil become active when adequate moisture is available. The J2 is a very active swimmer and has a relatively short period of infectivity (ca 3 days at 27 degrees C) (12). The preparasites swim near the surface of the water. When the nematode is within 1 cm of a mosquito host it swims klinotactically and secretes a small amount of adhesive material from the anterior portion of its body (16). This adhesive material appears to be important for attachment to the mosquito larva and for disinfesting the preparasite of contaminating microorganisms during penetration. Surface glycoproteins on the mosquito and the nematode also appear to be important for host-parasite recognition (8). The preparasite then begins odontostyle probing and penetration and esophageal secretions are introduced into the host causing temporary paralysis (16). After penetration, the parasite develops in the hemocoel of the young mosquito host for about 7-10 days (developmental time in the host is dependent upon the species of host, host nutrition, number of parasites, etc.). One

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molt occurs in the host larva and the parasite takes up most of its nourishment as monomeric or simple compounds through its cuticle (3,4). During the parasitic phase the host catabolizes most of its fat body reserves to feed the parasite which acts as a nutritional sink. The host sacrifices its long term growth and development (2) for short term activities such as osmoregulation (15), maintenance of hemolymph pH and energy balance (1,5) and basically burns its house to keep its guest warm. When the parasite emerges the host larva is severely depleted and dies shortly thereafter. The postparasite enters the soil environment at the bottom of the pool and molts to the adult, mates, and lays eggs, thus completing its life cycle. The postparasites are facultative anaerobes and can survive in conditions of low oxygenation (7).

CONTROL: R. culicivorax has been applied in inundative and inoculative control strategies and was marketed for a short time as "Skeeter Doom" by the Fairfax Biological Laboratories in Clinton Corners, New York (14). Unfortunately, "Skeeter Doom" is no longer commercially available for general use. An inundative strategy involves the mass rearing and release of the preparasitic stage as a biological pesticide. The mosquito environment is inundated with enough preparasites to effectively control the mosquito larvae and there is no long term expectation for establishment of the parasite. The applications are timed according to the host biology and density and other environmental factors. Results from experimental releases of preparasites for inundative control have not always been predictable and larval mortality has ranged from 0 to 90% (13,14). Also, Hominick and Tingley (6) argue that larval mortality is not a good measurement of the control potential of a treatment since high levels of larval mortality occur naturally. Failures in inundative releases of R. culicivorax may be linked to a variety of factors affecting the preparasitic stage. For example, abiotic factors such as temperature, ion toxicity, pH, pollution, and oxygen availability and biotic factors such as copepod predation are all known to significantly reduce the efficacy of the preparasite (13). Interestingly, the preparasitic stage is compatible with many insecticides at low concentrations and combined applications of the nematodes with specific doses of a chemical may be practical in the future (13,14).

Inoculative applications of \underline{R} . $\underline{\text{culicivorax}}$ involve the release of the postparasitic stage of the nematode for permanent colonization of an environment. Inoculative attempts have been made with \underline{R} . $\underline{\text{culicivorax}}$ and, although some recycling was demonstrated, the results were unpredictable and mosquito mortality in subsequent years was relatively low (13,14). Hominick and Tingley (6) argue that inoculative control by \underline{R} . $\underline{\text{culicivorax}}$ is theoretically implausible because of the long generation time of the parasite and host-parasite density-dependent constraints.

R. culicivorax can be easily mass cultured cost effectively in vivo using the mosquito host Culex pipiens (11). The cost utilizing these procedures can be as low as 7-10 cents per million preparasites (11). An aerial application system was used in Florida in 1977 at 27 psi to deliver 3,900 preparasites/square meter of pond surface for the partial control (more than 39%) of Culex and Anopheles mosquitoes (9). The cost of the nematodes for such an application could be as low as \$1.60 per acre. The in vitro cultivation of R. culicivorax has received attention in the past ten years but the nematode appears to be fastidious and a successful diet has not been developed (3,4).

Although \underline{R} . $\underline{\text{culicivorax}}$ cannot be considered the most promising biological control agent of mosquito larvae, it still has potential for use under special

ROMANOMERMIS CULICIVORAX

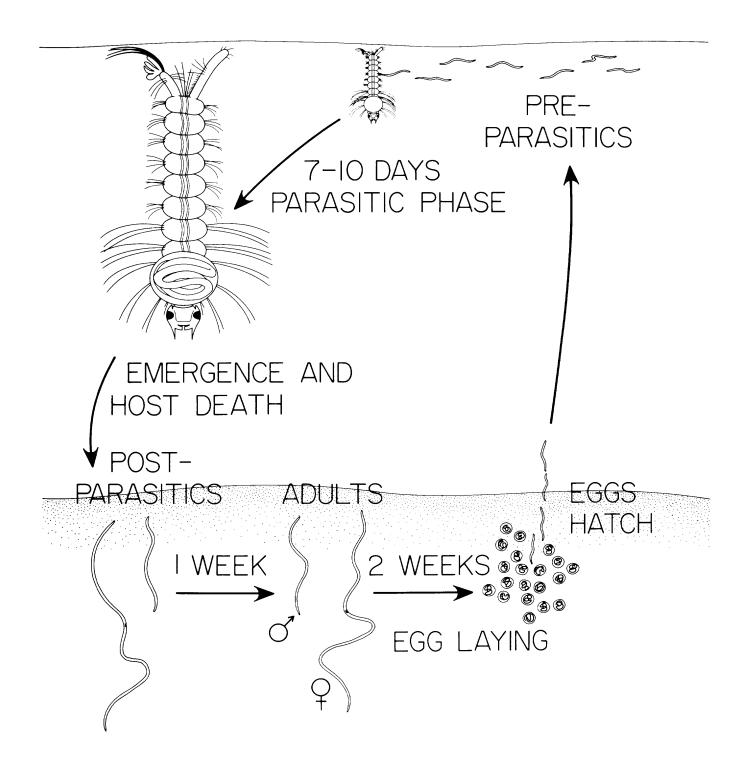


Figure 1. Generalized life cycle of <u>Romanomermis</u> <u>culicivorax</u>. (Reprinted with permission from Dr. E. G. Platzer, <u>Department of Nematology</u>, University of California, Riverside, CA 92521).

conditions. The inundative use of this nematode remains an attractive potential alternative to pesticides in large, unpolluted fresh water lakes and ponds near condominiums and tourist attractions in Florida.

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